Simultaneous Separation of Fat-Soluble Vitamins by Reversed-Phase HPLC Using a Cholesteryl Group Bonded Stationary Phase.

Toshi Ono[1], Tsunehisa Hirose[2], Kazuhiro Kimata[2]
[1]Nacalai USA, Inc., San Diego, United States, [2]Nacalai Tesque, Inc., Kyoto, Japan

Abstract

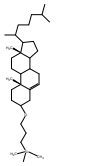
Vitamins are essential nutrients for human health, and can be classified as either water-soluble vitamins (B-complex and C) or fat-soluble vitamins (different forms of A, D, E and K). Several methods for the analysis of vitamins have described in the past. Reversed phase C18 columns are often used for the separation of water-soluble vitamins and the baseline separation can be achieved. However, the structural similarities between different forms of fat-soluble vitamins, such as vitamin D2 and D3, have posed chromatographic challenges to analysts interested in quantifying these vitamins within a single run. A cholesteryl group bonded stationary phase demonstrates superior selectivity of the analogs and allows simultaneous separation of 9 fat-soluble vitamins including D2 and D3.

Introduction

Although small amounts of fat-soluble vitamins are needed for human health, they generally pose a great risk for toxicity when consumed at very high levels. Therefore, it is important to assure the levels of fat-soluble vitamins in food products are accurate. This study demonstrated the characteristics and advantages of a novel cholesteryl group bonded stationary phase for simultaneous determination of fat-soluble vitamins, compared to the most commonly used sub-2 µm C18 stationary phases.

Stationary phase characteristics

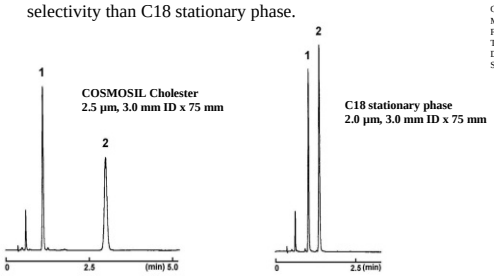
Silica Gel	High Purity Spherical Silica
Stationary Phase	Cholesteryl group
Average Particle Size	2.5 µm
Average Pore Size	130 Å
Surface Area	330 m^2/g
Carbon content	21%



Cholesteryl Group

Stationary phase characteristics: Shape selectivity 1

Figure 1: Cholesteryl stationary phase shows greater planarity selectivity than C18 stationary phase.



Column size : 3.0 mm I.D. x 75 mm
Mobile phase : 90% Methanol
Flow rate : 1.0 ml/min
Temperature : 40 ℃
Detection : UV 254 nm
Sample : 1. o-Terphenyl (0.05 µg)
2. Triphenylene (0.005 µg)

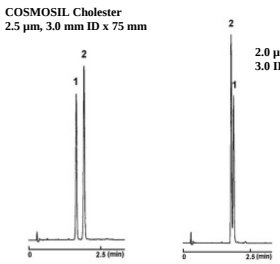
1. o-Terphenyl

2. Triphenylene

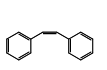
Stationary phase characteristics: Shape selectivity 2

Figure 2 : Separation of cis-trans isomers

Cholesteryl stationary phase offers improved resolution of geometric isomers.



Column size: 3.0mm I.D. x 75 mm
Mobile phase: Acetonitrile/ H_2O = 70/30
Flow rate: 1.0 ml/min
Temperature: 40 ℃
Detection: UV 254 nm
Sample: 1. *cis*-Stilbene (0.1 mg/ml)
2. *trans*-Stilbene (0.2 mg/ml)
Injection Vol. 1.0 µl



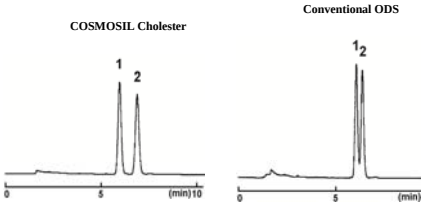
1. cis-Stilbene

2. trans-Stilbene

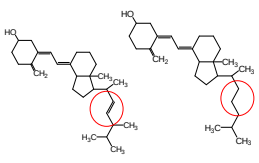
Stationary phase characteristics: Analog selectivity

Figure 3:

Cholesteryl stationary phase shows excellent selectivity for analogs with subtly different structures.



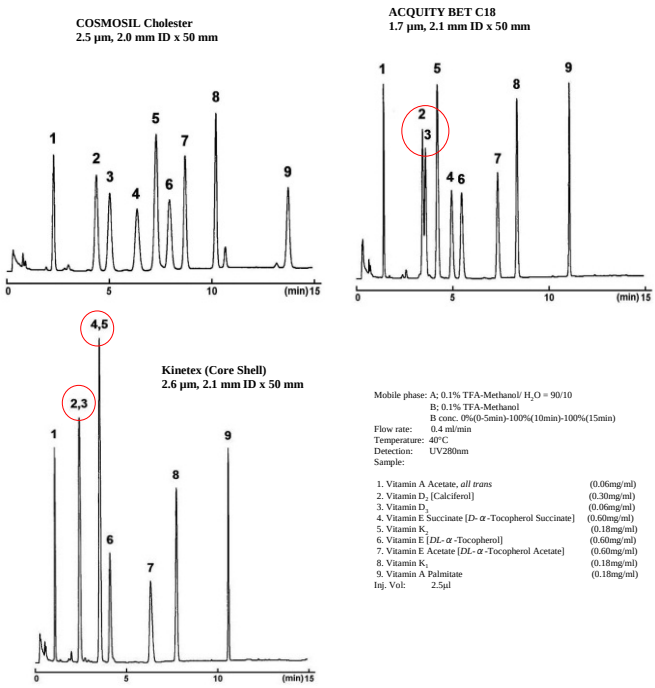
Column size : 4.6 mm I.D. x 150 mm
Mobile phase : 100% Methanol
Flow rate : 1.0 ml/min
Temperature : 30 ℃
Detection : UV 254 nm
Sample : 1.Vitamin D_3 (0.3 ug)
2.Vitamin D_2(0.1 ug)



Simultaneous Determination of 9 Fat-soluble Vitamins

Figure 4 : Separation of fat-soluble vitamins mixture

Cholesteryl stationary phase shows excellent resolution for all these 9 fat-soluble vitamins compared with 1.7 µm UHPLC C18 stationary phase and 2.6 µm core shell column.



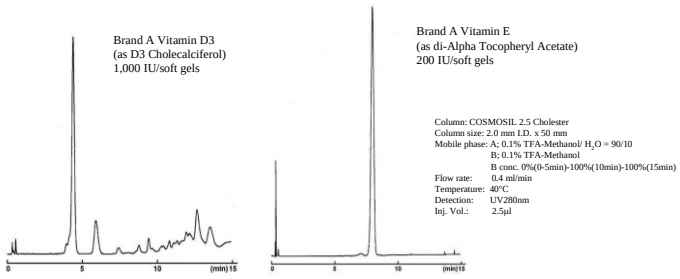
Mobile phase: A; 0.1% TFA-Methanol/ H_2O = 90/10
B; 0.1% TFA-Methanol
B conc. 0%(0-5min)-100%(10min)-100%(15min)
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV280nm
Sample:

1. Vitamin A Acetate, *all trans* (0.06mg/ml)
2. Vitamin D_2 [Calciferol] (0.30mg/ml)
3. Vitamin D_3 (0.06mg/ml)
4. Vitamin E Succinate [*D*- α -Tocopherol Succinate] (0.60mg/ml)
5. Vitamin K_2 (0.18mg/ml)
6. Vitamin E [*DL*- α -Tocopherol] (0.60mg/ml)
7. Vitamin E Acetate [*DL*- α -Tocopherol Acetate] (0.60mg/ml)
8. Vitamin K_1 (0.18mg/ml)
9. Vitamin A Palmitate (0.18mg/ml)

Inj. Vol: 2.5µl

Determination of Vitamin D and E in commercial supplement

Figure 5 : Determination of Vitamins in commercial products.



Column: COSMOSIL 2.5 Cholester
Column size: 2.0 mm I.D. x 50 mm
Mobile phase: A; 0.1% TFA-Methanol/ H_2O = 90/10
B; 0.1% TFA-Methanol
B conc. 0%(0-5min)-100%(10min)-100%(15min)
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV280nm
Inj. Vol.: 2.5µl

Conclusions

- COSMOSIL Cholester shows greater shape selectivity and better resolution for analogs with subtly different structures.
- Although sub-2 µm and core shell particles are used for very fast and high efficiency separation, conventional phase chemistries may not provide optimal separations for some compounds with structural similarities.
- COSMOSIL Cholester provides excellent resolution for the simultaneous separation of fat-soluble vitamins.